COMPARISON OF ANTHOCYANINS EXTRACTED WITH DIFFERENT SOLVENTS AND METHODS IN SELECTED BERRY FRUITS WITH AN AGRO-INDUSTRIAL POTENTIAL

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Abstract
Studies in anthocyanins and other naturally occurring pigments have made progress in the last years providing a valuable information about many classes of naturally occurring dietary phytochemicals present in foods. Their abundance in many red, purple, and blue color fruits has boosted their application as natural colorants in food, as well showing to be important to food quality and having possible health benefits due to their strong antioxidant activity. This paper aims to compare the anthocyanins content in selected berry fruits such as e blueberry, blackberry, raspberry and strawberry, their extraction affected by different solvents: methanol, ethanol, acetone different ratios 60 & 80 % (solvent/water v/v), and water, and the method of extraction (maceration, ultrasound assisted or not). The total anthocyanins content (TAC) was determined using pH differential method calculated as cyanidin-3-glucozide (C3G) mg/100 g fresh weight of sample (FW). Also, hunter color CIE values were determined, and L* a* b* resulted respectively from minimum to maximum values 23.08 to 29.45, 2.22 to 16.54, -0.37 to 7.11, where strawberry resulted to have highest values. The total anthocyanins ranged from 22.82 to 341.2 (C3G) mg/100 g FW, and the content of anthocyanins followed the order: blackberries>blueberries>raspberries>strawberries, which was affected by the method applied: ultrasound assisted>without ultrasound>maceration, and the solvent: MeOH 60 %> MeOH 80%> EtOH 80%> EtOH 60%> acetone 60%> acetone 80%> water. From this study may be concluded that selected fruits had very high anthocyanin content, with agro-industrial potential which may be utilized as natural colorants, and for food value addition. Further studies may be focused in finding innovative ways for their green and sustainable anthocyanins extraction.

Keywords: *anthocyanins, blackberry, blueberry, raspberry, strawberry*.

Introduction
Albania is placed in the Mediterranean Basin, offering enormous opportunities to grow many fruit crops, which have found spontaneous and wild forms of blackberries, raspberries (Rubus), strawberries (Fragaria) and vacciniums, and are part of a natural ecosystems (Kullaj et al. 2012). The term “red fruit” or “berry” is used to name the small fruits, sweet or bitter, juicy and intensely colored (usually red, purple or blue) that grow in wild bushes, can be eaten whole, and lack objectionable seeds. The most well-known red fruits are strawberry, raspberry, blueberry, blackberry, and cranberry. Berries, have recently attracted a lot of attention for their antioxidant properties, which are related to the high concentration of polyphenols present in them. In

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addition, their consumption worldwide has notoriously increased, and red fruits are nowadays not only consumed fresh but also used in cosmetics and dietary supplements (Hidalgo and Almajano 2017). Berry fruits are defined as functional food because of their protective and enhancing effects on health, affected by their various bioactive components, mainly high amounts of anthocyanins, which are water-soluble plant pigments responsible for the blue, purple, and red color of many plant tissues (Galván et al. 2014). Anthocyanins are common components of the human diet, founded in many foods and especially in berries while ellagitannins and anthocyanins are the most abundant phytochemicals in raspberries. There is increasing interest in the anthocyanin content of foods and nutraceuticals because of possible health benefits. There were more studies conducted on effect of processing and storage on changes and stability of colors of anthocyanins in foods such as fruits. Anthocyanin pigment content can also be a useful criterion in quality control and purchase specifications of fruit juices, nutraceuticals, and natural colorants. Studies in anthocyanins has been taking place to find the optimal extraction methods to obtain richly antioxidant products for a range of berries. Although conventional solvent extraction is the most widespread technique, new non-conventional methods have surfaced as environmentally friendly alternatives to the former method, such as ultrasound (Galván et al. 2012), microwave (Périno-Issartier et al. 2012), and pressure assisted extractions (Paes et al. 2014), applied alone or together with solvent use, to reduce the energy and solvent requirement.

This study shows that selected berry fruits have high anthocyanin content, and their utilization them as natural colorants or for food value addition may increase their agro-industrial potential and having an impact in Albanian agri-food sector.

Materials and Methods

For this study were selected berry fruits blueberry (*Vaccinium spp.*), blackberry (*Rubus spp.*), red raspberry (*Rubus idaeus*), and strawberry (*Fragaria ananassa*), collected randomly in 2021 in Tirana market in Albania, and immediately transported to the laboratory for further analyzation. Fruits were pre-selected with uniform maturity, shape, size, color, and free from defects.

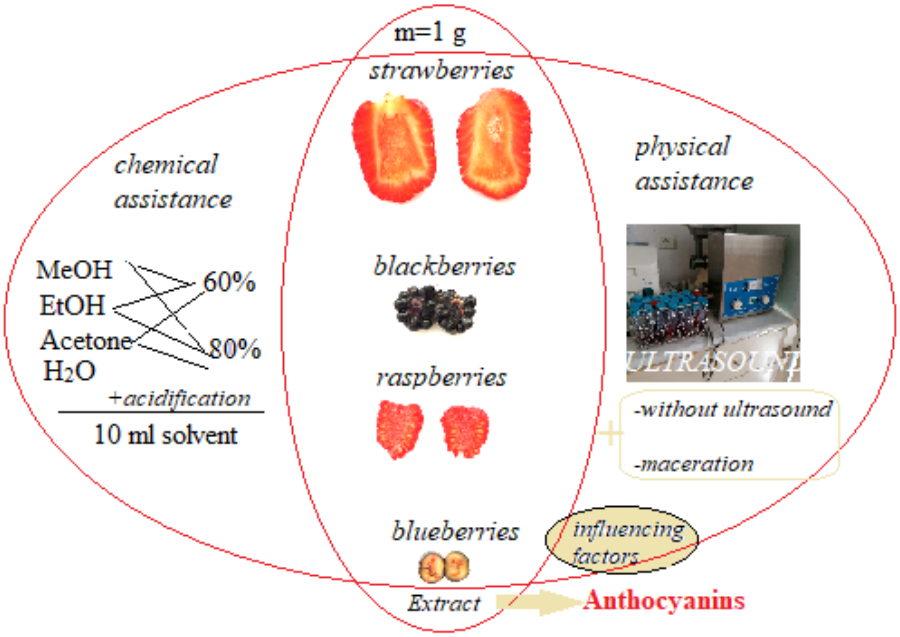

Fig. 1: Scheme of the extraction of anthocyanins from red fruits selected for this study

Extract preparation were performed according to Fig. 1, where 1 (±0.001) g of fruits pure was extracted by using different solvents (10 ml): methanol 60 and 80 % (solvent/water; v/v), ethanol 60 and 80 % (v/v), acetone 60 and 80 % (v/v), and water, all acidulated to enhance extraction, at a 1% amount, by using HCl. Different methods of extraction were applied: a) vortex for 1 min, ultrasound assisted for 15 min (40 KHz) 3500 rmp for 5 min, filtration, supernatant collection; b) same steps without ultrasound assisted; and c) maceration for 1 week at room temperature. The total anthocyanins content (TAC) was determined using a rapid measurement such as pH differential method (AOAC 2005.02), calculated as cyanidin-3-glucozide (C3G) mg/100 g fresh weight of sample (FW) as it is the most common compound in berries. pH measurements were done using pH meter (Lab 855), standardized with standard buffer solution at pH 4 and pH 7. Color measurement of blended sample were carried out using a portable colorimeter (model NH310) with the CIE L*, a*, b*.
Fruit's extracts preparation and other parameters determination were done at least in triplicates and presented as Mean ± standard deviation (SD).

Results and discussions
Result of this study indicate that selected berry fruits are rich in anthocyanins (Fig. 2) containing amounts respectively in blueberry (*Vaccinium spp*.) 29.74-317.65 mg C3G /100 g (FW), blackberry (*Rubus spp*.) 22.82-341.20 mg C3G /100 g (FW), raspberry (*Rubus idaeus*) 12.25-74.33 mg C3G /100 g (FW), and strawberry (*Fragaria ananassa*) 21.25-59.49 mg C3G /100 g (FW). From selected fruits blackberry reached the highest amount, with no significant differences compared to blueberry (7-23% differences in value), while with lesser amounts resulted in strawberry with no significant differences with raspberry (20-42% differences in value), while differences among blackberry, blueberry and strawberry, raspberry are approximately till 80%.

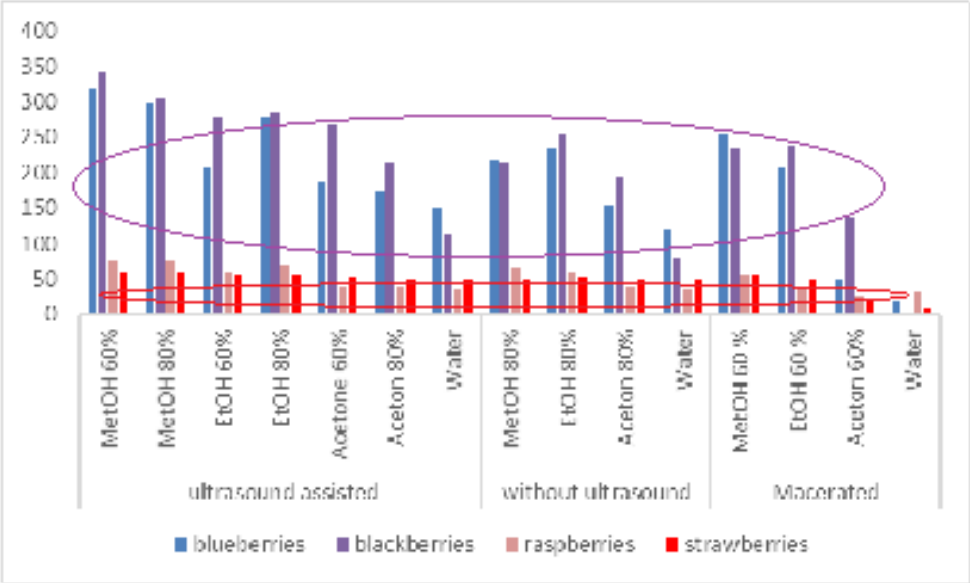

Fig. 2: Comparison of anthocyanins content in selected berry fruits

This variation of anthocyanins in berry fruits, can be explained with the dependence on genotype, cultivar (Häkkinen and Törrönen 2000), also the growing zone may affect, and from

our results may be concluded that blackberry and blueberry accumulate remarkably larger amounts of anthocyanins than strawberry, raspberry.

Although extraction techniques seem to have received much attention from researchers. The cultivars, season of harvesting, and geographic location of berries are important parameters that affect the content in the final extracts. Climate, sunlight exposure, water intake from plants, and ripening stage when berries are collected are very difficult to control. This is why the majority of researchers focus on the optimization of extraction techniques from different berries. Traditionally, there are used two main types of extraction of substances from a matrix: maceration by the release of them into a solvent, without heat application, over long periods of time and solvent extraction which works with the same mass transfer phenomena, but the use of a variety of solvents allows extraction of target components in a shorter time. To perform the extraction, there are three elements involved: the red fruit, the extraction method, which can be classified into the chemical or physical assistance category (or both), and the influencing factors, such as time and temperature (Hidalgo and Almajano 2017).

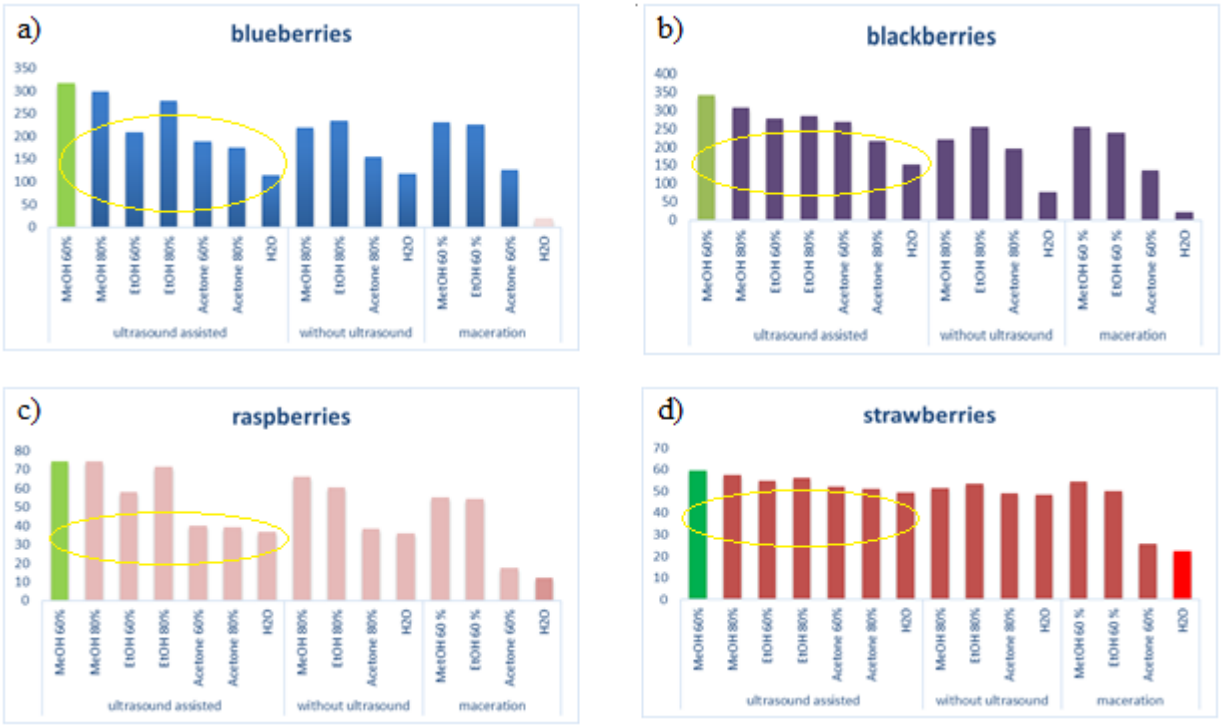

Fig 3: Comparison of extraction method effect on the anthocyanins

The comparison of these type of method in the anthocyanins content is given in the Fig. 3. It is noted that ultrasound assisted contributed more in the anthocyanin extraction, from 12 % to 28% compared to method without ultrasound assisted, and much more if it is compared to maceration from 27% to 79 %, and the same trend when comparison was made between the solvent extraction without ultrasound assisted and maceration, as it reached till 77% higher values of anthocyanins content. From results comparison, may be concluded that extractions performed using acidulated solvents such as methanol, ethanol, acetone, and water (Fig.3 a, b, c, d) affected the anthocyanins extraction by contributing in their content according to the order: ultrasound assisted> without ultrasound> maceration. Furthermore, we can recommend the ultrasound

assisted method at 40 kHz may be as useful to obtain extracts rich in anthocyanins, and its high efficiency in terms of time and solvent, also safe extraction of heat labile compounds is shown in other studies (Adjé et al. 2010). Ultrasound is considered as new non-conventional methods that have emerged as environmentally friendly alternatives to conventional method. However, should be mention that higher frequency and sonication time, without any added solvent showed a significant drop-in antioxidant activity due to the synergic effect of the ultrasound and temperature increase due to the high frequency applied (Golmohamadi et al. 2013).

From pH measurement it was seen that fruits of this study have a pH range 3.1-3.7. Berry fruit pH is significantly affected by fruits genotype, storage temperature, and the duration of storage (Palonen and Weber 2019). Anthocyanin pigments undergo reversible structural transformations with a change in pH manifested by different absorbance spectra. The oxonium form predominates at pH 1.0 while the hemiketal (colorless) form at pH 4.5. The pH-differential method is based on this reaction and allows accurate and rapid measurements of the total amount of anthocyanins, even in the presence of polymerized degraded pigment and other interfering compounds (Stoica *et al*. 2013). Expression of anthocyanin color is also pH dependent (Brouillard, 1982); during ripening of raspberry fruit, the concentration of organic acids decreases, while the concentration of anthocyanins increases along with color intensity (Krüger et al., 2011; Stavang et al., 2015).

Color is a major postharvest quality parameter for berry fruits and is genotype dependent. Samples color parameters were determined using CIE L* a* b*, and values resulted respectively 23.08-29.45, 2.22-16.54, and -0.37 to 7.11. The L* (lightness) value range is from 0 = black to 100 = white, the a* range is negative for green to positive for red, and b* values are negative for blue to positive for yellow (Palonena and Weber 2019). Among berry fruits strawberry resulted to have highest lightness (29.45), with a higher positive a* (16.54) value indicating redder, with a higher positive b* value too. Whereas the fruit with the lowest value resulted blackberry. Fruit color is a major component of fruit quality and is directly connected to the concentration and composition of anthocyanins in the fruit (García-Viguera et al., 1998).

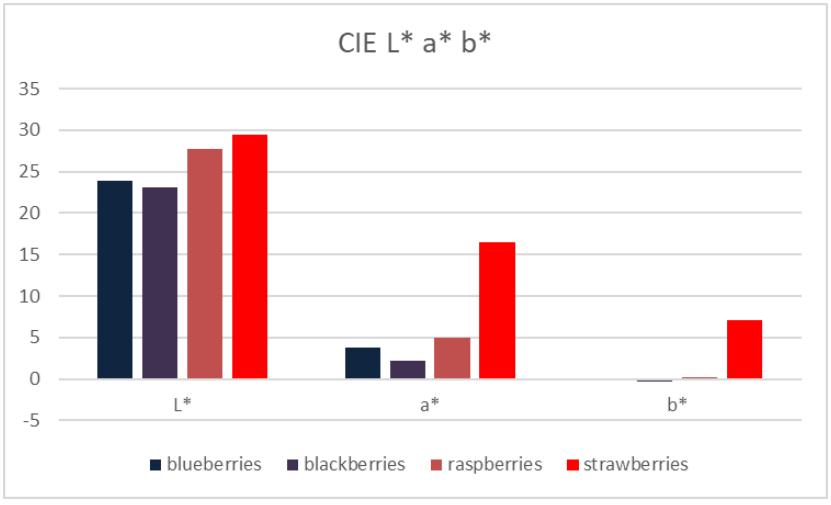

Fig. 4: CIE L* a* b* values of selected berry fruits

Anthocyanins are water soluble pigments (phenolic glucosides) in the flavonoid group localized in cell vacuoles and often in epidermal layers of fruit. Intense red-purple colors are typical of anthocyanins, and total anthocyanin content is correlated with visible berry fruits color, which is in accordance with similar studies (Anttonen and Karjalainen, 2005; Weber et al., 2008).

Lightness (L* value), a*, b* are highly significantly affected by genotype, storage temperature and the duration of storage. According to the numbering system used by the Codex Alimentarius Commission, anthocyanins (any anthocyanin-derived colorant) are listed as a natural colorant by the European Union (EU) legislation as product E163, so considering the high content of anthocyanins in the fruits of this study may be utilized as good source of natural colorant and Anthocyanins are interesting pigments regarding their chromatic features, display a wide range of attractive colors, are water-soluble, have health-promoting effects, and may be used for sugar confectionary, dairy products, ice creams, etc., (Mateus and Freitas 2008).

At the Fig. 5 (a, b, c, and d) are given comparison of solvents effect on anthocyanins of berry fruits. It is noted that methanol-water mixture is more efficient than other solvent systems in extracting of anthocyanins. Two ratios water–solvent was tested and the extraction yields of anthocyanins obtained with 60% methanol at room temperatures were about till 28% higher than the yields of extraction using 60% ethanol, till 46 % higher compared to 60 % acetone, and till 67 % compared to water extracts; also, the 60% methanol solvent yielded extraction till 10 % higher compared to 80% methanol. The 80% ethanol yielded extraction till 20 % higher compared to 60% ethanol, 31 % compared to 60% acetone, and 59 % compared to water extracts. The 60% acetone yielded extraction till 19.5 % higher compared to 80% acetone, and 58 % compared to water extracts. Numerous studies have tested the effectiveness of different solvents for the extraction and recovery of antioxidant compounds, and ethanol has been shown to be the best when comparing it with water, acetone, hexane, ethyl acetate (Galván D'Alessandro et al. 2012; Benzie and Strain 1996). In this study effectiveness of solvents followed the order: MeOH 60%> MeOH 80 %> EtOH 80%> EtOH 60%> acetone 60%> acetone 80%> H_2O.

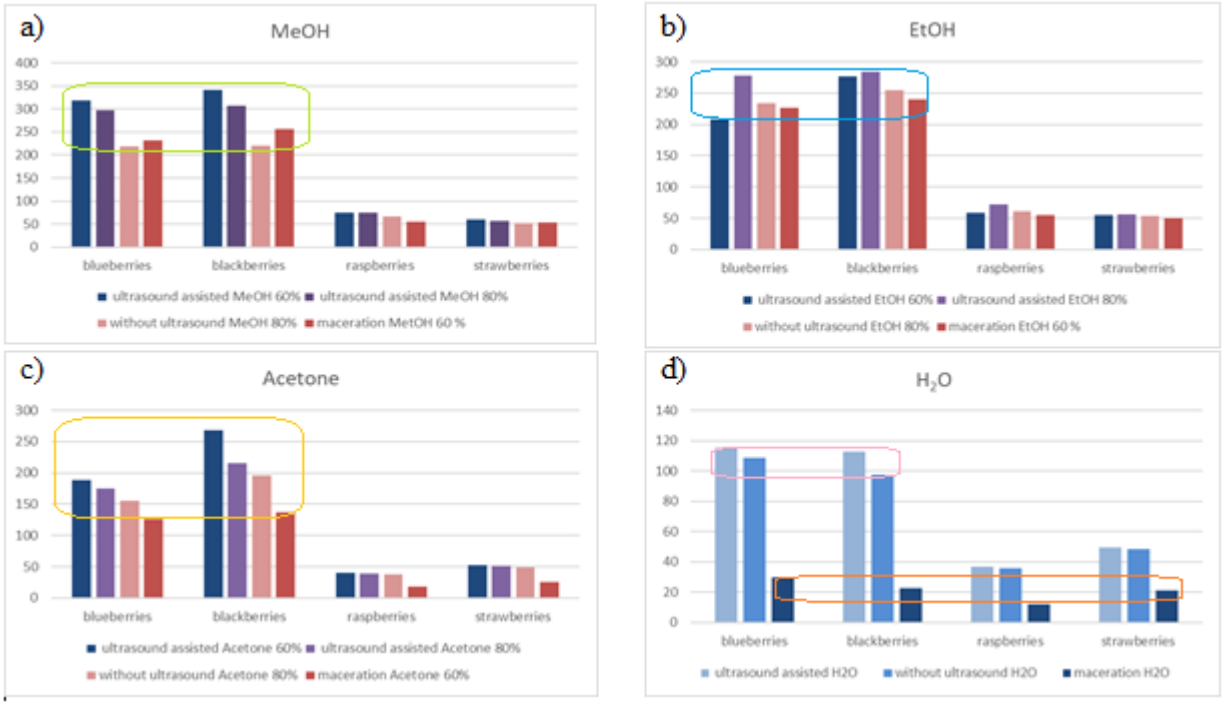

Fig. 5: Comparison of different solvent effect on the anthocyanins

Conclusions

This paper compared the anthocyanins content in selected berry fruits such as e blueberry, blackberry, raspberry and strawberry, their extraction affected by different solvents: methanol, ethanol, acetone different ratios 60 & 80 % (solvent/water v/v), and water, and the method of extraction (maceration, ultrasound assisted or not). From selected fruits blackberry reached the highest amount, and was followed the order: blackberries>blueberries>raspberries>strawberries. There were not significant differences compared to blueberry (7-23% differences in value), while with lesser amounts resulted in strawberry with no significant differences with raspberry (20-42% differences in value), while differences among blackberry, blueberry and strawberry, raspberry are approximately till 80%. From the comparison of extraction methods was noted that ultrasound assisted contributed more in the anthocyanin extraction, from 12 % to 28% compared to method without ultrasound assisted, and much more if it is compared to maceration from 27% to 79 %, and the same trend when comparison was made between the solvent extraction without ultrasound assisted and maceration, as it reached till 77% higher values of anthocyanins content. The extraction method affected anthocyanins by contributing in their content according to the order: ultrasound assisted> without ultrasound> maceration, so may be recommended that ultrasound as new non-conventional methods, environmentally friendly, and as good alternative to conventional method. In this study effectiveness of solvents by contributing anthocyanins content followed the order: MeOH 60%> MeOH 80 %> EtOH 80%> EtOH 60%> acetone 60%> acetone 80%> H_2O. From this study may be concluded that selected fruits had very high anthocyanin content, and considering the high content of anthocyanins, blueberry, blackberry, raspberry and strawberry may be utilized as good source of natural colorant with agro-industrial potential, also for food value addition. Further studies may be focused in finding innovative ways for their green and sustainable anthocyanins extraction and their importance to quality attributes of food, and development of functional foods.

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